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OBSERVATIONS ON THE DURAL NERVE FIBERS BY MEANS OF THE CONTINUOUS INTRAVENOUS PERFUSION OF METHYLENE BLUE

by

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INTRODUCTION

Since Ehrlich[3] reported the vital staining of the nervous system with methylene blue in 1886, various neural elements including the dural nerve fibers have been stained and investigated with the dye by Huber[9], Wreden[16] and Dowgjallo[2] etc. Huber[9] studied the dural nerve fibers in living animals, such as dogs, rabbits, and cats, by injection of methylene blue into the carotid artery. In the studies of Wreden[16], the dura of cats, dogs and horses was soaked in methylene blue solution of 37°C in temperature for about one hour soon after the death of animals. Dowgjallo[2] investigated the dural nerve fibers of mammals by the following complicated method. The blood was driven out from intracranial vessels with the instillation of the special solution into the aorta. After the brain was removed, the basis of the skull was dissected and immersed in the dye solution for 5～6 hours at the temperature of 30°C.

I have reexamined in detail the report of Feindel et al[5] concerning the vital staining of the central nervous system with methylene blue for some years, and it has been recognized that a part of the dye perfused into the rabbit's vein penetrated per diapedesin into the surrounding tissue in the vicinity of the injection point. Accordingly, in the skin of the rabbit's ear near the point of injection, the nerve fiber bundles were stained blue just as Woollard et al[15] and Weddell et al[14] investigated by the local injection of dilute dye solution (Fig. 1). Being given a hint from these findings, a piece of the dura of the animals was placed on an object glass and investigated. Our findings on the dural nerve fibers thus studied were nearly in accord with the descriptions in literature up to the present. In 1947, Feindel et al[5] reported that the dural nerve fibers, too, could be stained by the continuous intravenous perfusion of methylene blue. But the report had no systematic studies concerning the dura and no definite descriptions even of the procedures in microscopical examination. Therefore, our experimental procedures, observations and some discussions will be reported in the following.

MATERIALS AND METHODS

Experiments were performed using 15 rabbits weighing 1～3 kg and 5 dogs

weighing 3～7 kg.

Perfusion technique of the dye generally followed the original method (intravenous drip technique) of FEINDEL et al[5]. As the dye Methylenblau f. med. Zwecke (MERCK) was used. In each experiment, 0.5% solution of the dye was newly made up in physiological saline or 5% glucose solution. The dye solution was perfused into the marginal ear vein of rabbits and into the saphenous vein of dogs at the rate of 4～20 drops per minute. The dye perfusion was continued for more than 30 minutes in rabbits and for more than 3 hours in dogs.

After the animals died or while the animals were alive, dural pieces of 1～3 cm^2 in size covering various portions of the brain were investigated, dividing into two groups, i. e. the dura in the convexity and that in the basis.

In order to fix the dye a piece of dura was immersed in 6～8% solution of ammonium molybdate, made up in physiological saline and newly prepared in each experiment, for 1～4 hours at the temperature of 0～2°C in the refrigerator. After washing the piece thoroughly for 20～30 minutes with physiological saline and then with distilled water, it was dehydrated with alcohol or by desiccation (placed in an incubator at the temperature of 37°C for about 20 minutes) without formalin fixation.

After the dural piece was dehydrated, it was cleared with xylol, mounted in balsam and then submitted to the investigation.

RESULTS

It could always be recognized that many nerve fibers, thick medullated and thin non-medullated, were clearly stained blue in the piece of dura. The stainability of the dural nerve fibers were much better in rabbits than in dogs. Most of them ran in bundles along the blood vesseels (Fig. 2) and some of them were considered to distribute on the vessel walls by ramifications (Fig. 3). Single thick medullated nerve fiber having no relation to vessels was also found (Fig. 4, 5; Fig. 12 in "Evaluation of the Methylene Blue Vital Staining of the Brain with Intravenous Drip Technique" by the author[1]). Moreover bundles consisting of many thin medullated nerve fibers could be found (Fig. 4). These nerve fibers distributed in the dura forming a network with each other (Fig. 6, 7). In a typically stained thick medullated fiber, the myelin sheath was stained light blue and nodes of RANVIER deep blue (Fig. 4; Fig. 12 in "Evaluation of the Methylene Blue Vital Staining of the Brain with Intravenous Drip Technique" by the author[1]). Special terminal corpuscles could not be recognized, but terminal branches were found here and there (Fig. 8, 9, 10).

More nerve fibers were found in the dura of the cranial basis than in the convexity. Accordingly, in the dura of the basis the network of nerve fibers was finer and nerve bundles were thicker than in the convexity. Namely, the thickest nerve bundles in the dura of the convexity were only 10～15μ in thickness (Fig. 11), while those of the basis were 40～50μ in thickness (Fig. 3, 4). In a few animals the dural nerve fibers of the spinal cord and the cerebellar tentory were also

investigated and the distribution of nerve fibers was relatively dense as in the dura of the basis.

[SUPPLEMENT] OBSERVATIONS OF OTHER THIN TISSUES

Other thin tissues such as the periosteum, the fascia, the pleura, the peritoneum and the mesentery, were also investigated with the same procedures. But, few nerve bundles could be recognized in the periosteum of the skull at the base of the ear on the injected side where the blue coloration was visible macroscopically (Fig. 12).

DISCUSSION

According to STÖHR[11], KOPSCH[9], TRAUM[13], DOWGJALLO[2], WREDEN[16] and HUBER[8], the dural nerve fibers have been described as follows. (i) Most of them run along blood vessels. (ii) The dura mater receives its nerves principally from the trigeminal, and partially from the glossopharyngeal, vagal, accessory, hypoglossal and from the sympathetic nerves. (iii) They are divided into two groups, i, e. the vasomotor nerves and nervi proprii (the proper dural nerves), the latter being considered as the sensory ones.

In our preparations, many nerve bundles running along blood vessels, some of them being considered to distribute on the vessel wall by ramifications, are well in accord morphologically with the vasomotor nerves. On the other hand, the medullated nerve fibers having no relation to vessels are considered to be the sensory ones. The myelin sheaths of thick medullated nerve fibers are stained light blue, as WEDDELL et al[14] have recognized in the skin of rabbit's ear by local injection of the dye. The features that nodes of RANVIER are stained deep blue and sometimes show the appearance like a cross coincide with the description of WEDDELL et al,[14] of HUBER[8] concerning sensory nerve fibers of the pia mater and of TAUCHI[12] on the supravital staining of nerve fibers. Although TRAUM[13] has recognized numerous nerve fibers in the dura of the middle cranial fossa, there are, it seems, few reports, that more nerve fibers and thicker nerve bundles can be found in the dura of the cranial basis than in the convexity. In the pia mater, however, STÖHR[10] and HASSIN[7] etc. have demonstrated more nerve fibers in the cerebral basis. From our observations similar findings are recognizable in the dura as in the pia.

As the special termination of the proper dural nerve, the fine tree-like branches and the skein-like end bulbs have been described by STÖHR[11], "knopfförmige Anschwellung" by KOPSCH[9], "Nervenendbäumchen" by TRANM[13], "Zwischenplättchen" and "Zwischennetzchen" by DOWGJALLO[2], "baumförmige Endverzweigung" and "spezieller Endapparat" by WREDEN[16]. Generally speaking, two kinds of the nerve termination, i. e. the terminal branches and the terminal corpuscles, may be recognized in the dura. In our experiments, we have been able to recognize only the terminal branches.

Contrary to our clinical experiences in surgery that the pleura and the peritoneum are far more sensitive than the dura, nerve fibers are clearly stained in the dura and never in the pleura, the peritoneum and in the mesentery in our experi-

ments. This fact makes us consider that distribution of nerve fibers may be looser in the other thin tissues than in the dura. It may be assumed that the fact is partially due to the differences in the distribution density of blood vessels and the peculiarity in the circulatory system of the head, i. e. the venous system inside and outside of the skull has mutual anastomoses through the emissary. The presence of the emissary may also explain the findings that the dural nerve fibers of rabbits are better stained with methylene blue than those of dogs. The dye solution has been perfused into the marginal ear vein in rabbits, so the internal pressure of the marginal vein increases at first, and then of the retromandibular vein. Accordingly, a part of the dye solution must be directly perfused into the dural venous sinus through the emissary. As the result of partial penetration of the dye solution into the dural tissue per diapedesin from the venous sinus, the dural nerve fibers may be stained clearly in rabbits. After the dye perfusion, the dural venous sinus of rabbits is evidently colored deep blue. The difference in the stainability between rabbits and dogs may also be, of course, due to the difference in per kg dosage of the perfused dye between the two animals.

Because the degenerating features of the nerve fibers can be recognized only in the dura immersed in the dye fixative for long duration (more than 24 hours), we may consider that the dural nerve fibers in other pieces were capable of being investigated in nearly normal conditions in our experiments. In a dural piece of one rabbit immersed in the dye fixative for such a long duration, rosary-like rhomboid swellings, similar to the degenerating features, have been recognized in a single medullated nerve fiber. This rosary-like appearance of nerve fiber bears a close resemblance to the finding that GLEES et al[6] have recently considered to be an artefact in preparations stained with silver impregnation. The investigation materials must not be immersed in the dye fixative for long period.

Furthermore, the staining preparations by our method often fade away after a long while. Usually it may be difficult to preserve the preparations for more than about six months. To explain the reason we may suggest that no formalin fixation has been done in our experiments.

CONCLUSION

1) The dural nerve fibers of rabbits and dogs were stained with methylene blue by the intravenous drip technique.

2) Many nerve bundles, medullated and non-medullated, running along blood vessels were recognized in the dura. Besides, medullated nerve fibers having no relation to blood vessels (perhaps sensory ones) were found. These nerve fibers distributed in the dura forming a fine network with each other.

3) Two kinds of the nerve termination, i. e. the terminal branches and the terminal corpuscle, have been hitherto recognized in the dura, while, only the terminal branches were found in our experiments.

4) Not only in the pia but also in the dura more nerve fibers and thicker nerve bundles could be recognized in the cranial basis than in the convexity.

5) Other thin tissues than the dura, such as the periosteum, the fascia, the pleura, the peritoneum and the mesentery, were investigated with the same procedures. But no nerve fibers could be stained.

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* Written in Japanese.

和 文 抄 録

メチレン青静脈内点滴注入法による硬膜神経線維の観察

京都大学医学部外科第一講座（指導：荒木千里教授）
大学院学生 安 藤 協 三

1) メチレン青静脈内点滴注入法によつて，家兎及び犬の硬膜神経線維を観察した．本法（点滴注入）による硬膜神経線維の観察は従来あまり行われていない．

2) 有髄性及び無髄性の神経線維束が主として血管と相伴つて走り，其他血管とは無関係に走る有髄神経線維（恐らくは知覚性）の存在をも認め，之等は相互に連絡して微細な網目を作つている．此の所見は在来の文献に見られる記載と略々一致する．

3) 硬膜の神経終末は終末分枝及び終末小体とに大別されるが，観察例数が少い為か，本法では終末分枝のみを認めた．

4) 柔膜のみならず，硬膜においても，頭蓋底部は脳穹隆部に比較して，神経線維の分布は密であり，且つ太い神経線維束が認められた．

5) 同様操作を以て少数例で，胸膜・腹膜・腸間膜・筋膜・骨膜をも観察したが，神経線維は全然染色されなかつた．

6) 本実験の結果について若干の考察を加えると共に2,3の注意事項を附記した．

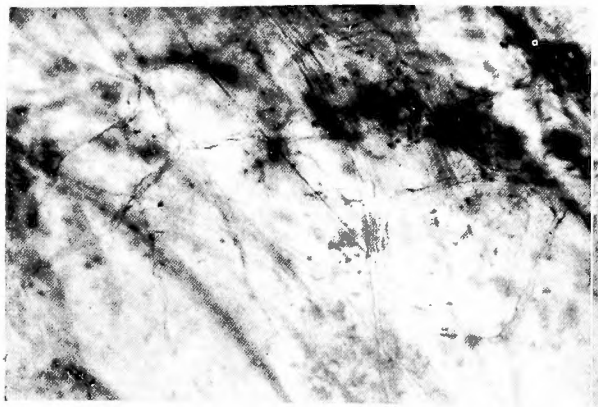

Fig. 1 : Cutaneous nerve fibers in the rabbit's ear (×100).

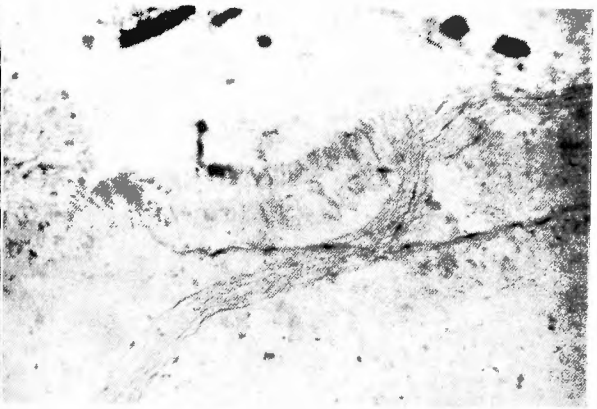

Fig. 4 ; Thick single medullated nerve fiber having no relation to the blood vessels. Nodes of RANVIER were stained deep blue and myelin sheaths were stained light blue (in the dura covering the cerebral basis of rabbit) (×100).

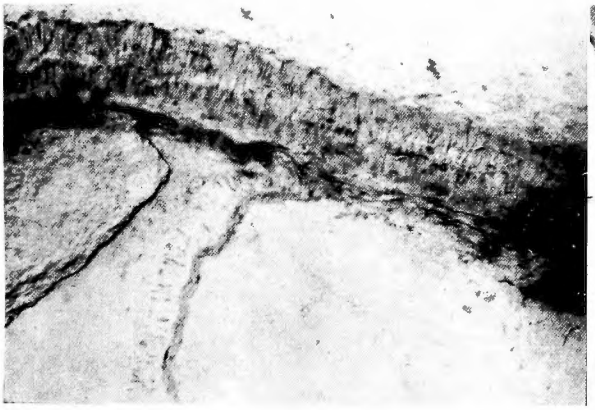

Fig. 2 : Dural nerve bundles of rabbit running along the blood vessels in the basis of the skull (×100).

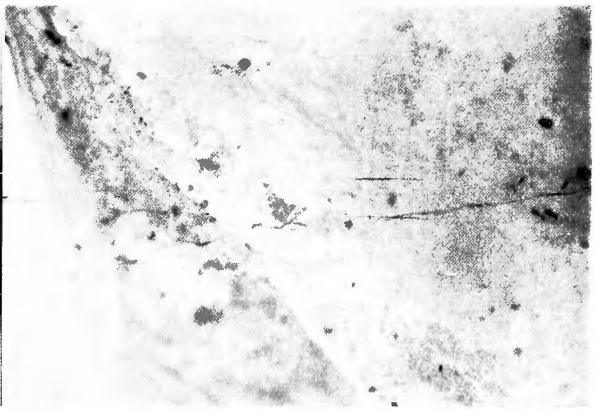

Fig. 5 : Thin single medullated nerve fiber. Nodes of RANVIER were stained deep blue (in the dura covering the convexity of the rabbit's brain) (×100).

Fig. 3 : Dural nerve bundles of rabbit in the basis of the skull. Thick bundles having no relation to the blood vessels. Thin bundles running along the blood vessels distributed on the vessel wall by ramifications (×100).

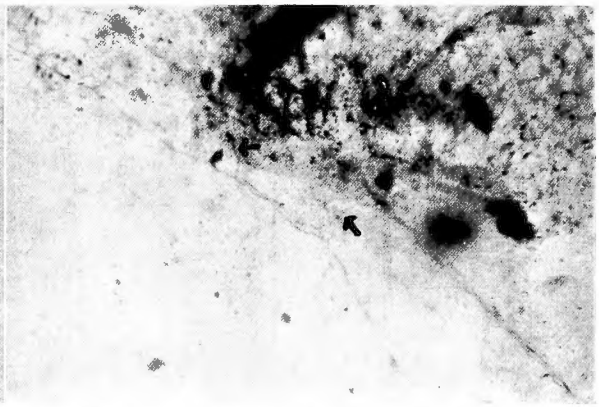

Fig. 6 ; The dura in the convexity of the rabbit's brain. Two nerve bundles running obliquely with anastomoses (↑) (×100).

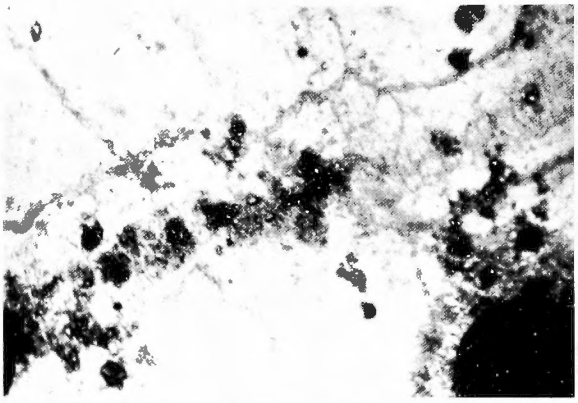

Fig. 7 ; Network of nerve fibers recognized in the dura covering the cerebral basis of the rabbit's brain. Ramifications on the vessel wall could be found (×100).

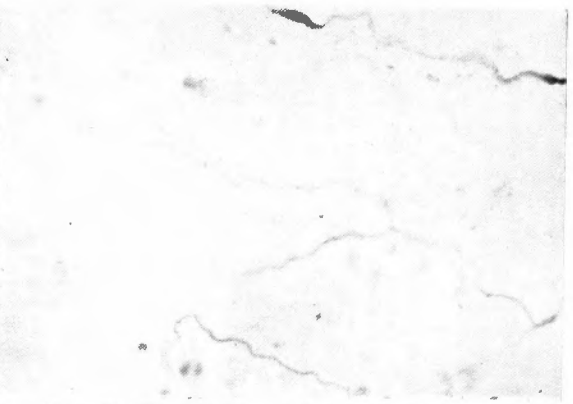

Fig. 10 : Terminal branches in the dura covering the cerebral basis of rabbit (×900).

Fig. 8 : Terminal branches in the dura covering the cerebral convexity of rabbit (×400).

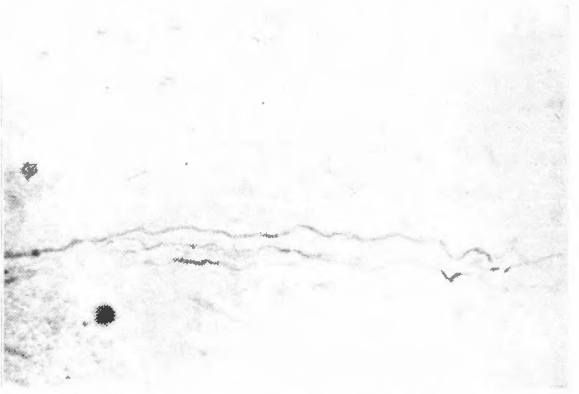

Fig. 11 : The thickest nerve bundle being recognizable in the dura in the parietal region of the rabbit's brain (×400).

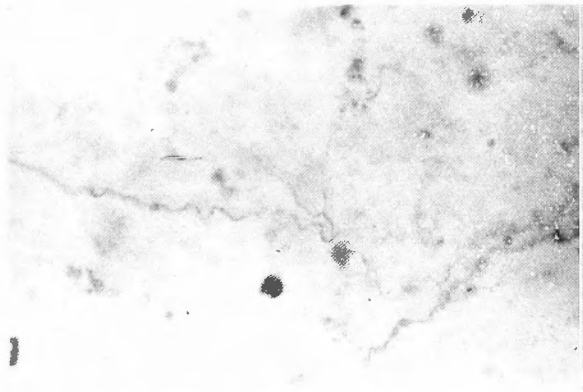

Fig. 9 : Terminal branches in the dura covering the cerebral basis of rabbit (×400).

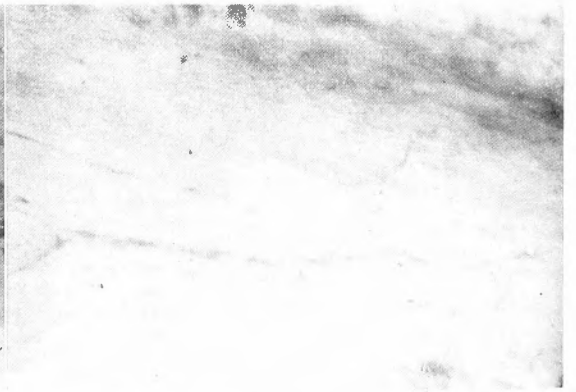

Fig. 12 : Nerve fibers recognized in the cranial periosteum of rabbit (×200).